

## CLAIMS

What is claimed:

- 1           1. A method for preparing an tracer composition  
2   comprising:  
3           obtaining a  $^{13}\text{C}$  labeled Krebs cycle metabolite  
4   precursor that will produce an analyte;  
5           obtaining a deuterium source;  
6           wherein gluconeogenesis is measured from a subject  
7   that was provided the precursor and the deuterium source,  
8   and produced the analyte, by comparison of the relative  
9   nuclear magnetic resonance profiles of the labeled  
10   components in the analyte.
- 1           2. The method of claim 1, wherein the analyte is  $^{13}\text{C}$ -  
2   glucose.
- 1           3. The method of claim 1, wherein the precursor is  
2   glucose, lactose, lactate or alanine.
- 1           4. The method of claim 1, wherein the deuterium  
2   source is deuterated water.
- 1           5. The method of claim 1, wherein the analyte is  
2   glucose deuterated in the 2, 5 and 6 positions, and any

3 transformation that maintains the 2,5 and 6 positions in  
4 relation to one another.

1 6. The method of claim 1, wherein the analyte is (1-  
2 6  $^{13}\text{C}_2$ )-glucose.

1 7. The method of claim 1, wherein the water is  $\text{D}_2\text{O}$ .

1 8. The method of claim 1, wherein the flux is  
2 measured from blood, urine or tissue extracts.

1 9. The method of claim 1, wherein the analyte is  $^{13}\text{C}$ -  
2 labeled glucose with the label at the 2 or 5 positions,  
3 or at both positions.

4 10. The method of claim 9, wherein the metabolite is  
5 a transformation of the labeled glucose containing the  
6 labeled 2 position, or the labeled 5 position, or both.

1 11. The method of claim 1, further comprising the  
2 step of adding  $^{13}\text{C}_3$ -propionate.

1 12. The method of claim 1, wherein the Krebs cycle  
2 precursor is selected from the group consisting of  
3 pyruvic acid, acetic acid, acetoacetic acid, beta-

4 hydroxybutyric acid, a Krebs cycle pathway metabolite,  
5 and mixtures thereof.

1 13. The method of claim 1, wherein the analyte is  
2 selected from the group consisting of pyruvic acid,  
3 acetic acid citric acid, isocitric acid, cis-aconitic  
4 acid, 2-ketoglutaric acid, succinic acid, fumaric acid,  
5 malic acid, oxaloacetic acid, and mixtures thereof.

1 14. A method for preparing an tracer composition  
2 comprising:  
3 obtaining a deuterium source;  
4 wherein gluconeogenesis is measured from a subject  
5 that was provided the deuterium source, and produced an  
6 analyte, by comparison of the relative nuclear magnetic  
7 resonance profiles of the deuterium components in the  
8 analyte.

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1 15. The method of claim 14, wherein the deuterium  
2 source is deuterated water.

1        16. The method of claim 14, wherein the analyte is  
2 glucose deuterated in the 2, 5 and 6 positions, and any  
3 transformation that maintains the 2,5 and 6 positions in  
4 relation to one another.

1        17. The method of claim 14, wherein the analyte is  
2 (1-6  $^{13}\text{C}_2$ )-glucose.

1        18. The method of claim 14, wherein the flux is  
2 measured from blood, urine or tissue extracts.

1        19. The method of claim 14, wherein the analyte is  
2 selected from the group consisting of pyruvic acid,  
3 acetic acid citric acid, isocitric acid, cis-aconitic  
4 acid, 2-ketoglutaric acid, succinic acid, fumaric acid,  
5 malic acid, oxaloacetic acid, and mixtures thereof.

1        20. A method for preparing an isotopic metabolic  
2 flux tracer composition comprising:

3        providing a  $^{13}\text{C}$  labeled Krebs cycle metabolite  
4 precursor to a subject to produce an analyte;  
5        obtaining a sample from the subject; and

6 measuring the nuclear magnetic resonance of the  
7 labeled tracers to determine the rate of gluconeogenesis.

1 21. The method of claim 20, wherein the analyte is  
2  $^{13}\text{C}$ -glucose.

1 22. The method of claim 20, wherein the analyte is  
2 glucose labeled with  $^{13}\text{C}$  at positions 1 through 6, or  
3 combinations of two or more at any position.

1 23. The method of claim 20, wherein the analyte is  
2 (1-6  $^{13}\text{C}_2$ )-glucose.

3 24. The method of claim 20, wherein the sample is  
4 from blood, urine or tissue extracts.

5 25. The method of claim 20, further comprising the  
6 step of providing the subject with  $^{13}\text{C}_3$ -propionate.

1 26. The method of claim 20, wherein the Krebs cycle  
2 precursor is selected from the group consisting of  
3 pyruvic acid, acetic acid, acetoacetic acid, beta-  
4 hydroxybutyric acid, a Krebs cycle pathway metabolite,  
5 and mixtures thereof.

1        27. The method of claim 20, wherein the analyte is  
2        selected from the group consisting of pyruvic acid,  
3        acetic acid citric acid, isocitric acid, cis-aconitic  
4        acid, 2-ketoglutaric acid, succinic acid, fumaric acid,  
5        malic acid, oxaloacetic acid, and mixtures thereof.

1        28. The method of claim 20, wherein the  $^{13}\text{C}$  Krebs  
2        cycle precursor is provided orally.

1        29. A method for measuring metabolic flux in a  
2        sample using an isotopic metabolic flux tracer  
3        composition comprising:  
4        providing the sample with a  $^{13}\text{C}$  Krebs cycle  
5        precursor,  $\text{D}_2\text{O}$  and acetaminophen;  
6        obtaining an analyte from the sample; and  
7        measuring the relative amounts of acetaminophen  
8        glucuronide and phenylacetylglutamine in the analyte using  
9        nuclear magnetic resonance.

10       30. The method of claim 29, wherein the precursor is  
2        $^{13}\text{C}$ -glucose.

1           31. The method of claim 29, wherein the precursor is  
2 glucose labeled with  $^{13}\text{C}$  at positions 1 through 6, or  
3 combinations of two or more at any position.

1           32. The method of claim 29, wherein the precursor is  
2 (1-6  $^{13}\text{C}_2$ )-glucose.

1           33. The method of claim 29, wherein the sample is  
2 from blood, urine or tissue extracts.

1           34. The method of claim 29, further comprising the  
2 step of providing the subject with  $^{13}\text{C}_3$ -propionate.

1           35. The method of claim 29, wherein the Krebs cycle  
2 precursor is selected from the group consisting of  
3 pyruvic acid, acetic acid, acetoacetic acid, beta-  
4 hydroxybutyric acid, a Krebs cycle pathway metabolite,  
5 and mixtures thereof.

1           36. The method of claim 29, wherein the Krebs cycle  
2 precursor is selected from the group consisting of  
3 pyruvic acid, acetic acid citric acid, isocitric acid,  
4 cis-aconitic acid, 2-ketoglutaric acid, succinic acid,  
5 fumaric acid, malic acid, oxaloacetic acid, and mixtures  
6 thereof.

1           37. The method of claim 29, wherein the  $^{13}\text{C}$  Krebs  
2   cycle precursor and  $\text{D}_2\text{O}$  are provided orally.

1           38. The method of claim 29, wherein the  $^{13}\text{C}$  Krebs  
2   cycle precursor and  $\text{D}_2\text{O}$  are provided to a mammal.

1           39. The method of claim 29, wherein the  $^{13}\text{C}$  Krebs  
2   cycle precursor and  $\text{D}_2\text{O}$  are provided to a human.

1           40. A reagent kit for use in effecting a  
2   simultaneous assay for gluconeogenesis in a sample, said  
3   reagent kit comprising:  
4   a  $^{13}\text{C}$  labeled Krebs cycle precursor; and  
5   a labeled water tracer.

1           41. The reagents of claim 40, wherein the Krebs  
2   cycle precursor is  $^{13}\text{C}$ -glucose.

1           42. The reagents of claim 40, wherein the Krebs  
2   cycle precursor is  $(1-6\ ^{13}\text{C}_2)$ -glucose.

1           43. The method of claim 40, wherein the Krebs cycle  
2   precursor is glucose labeled with  $^{13}\text{C}$  at positions 1  
3   through 6, or combinations of two or more at any  
4   position.



1           44. The reagents of claim 40, wherein the water  
2   tracer is D<sub>2</sub>O.

1           45. The reagents of claim 40, wherein the Krebs  
2   cycle precursor is <sup>13</sup>C<sub>2</sub>-labeled glucose.

3           46. The reagents of claim 40, further comprising  
2   <sup>13</sup>C<sub>3</sub>-propionate.

1           47. The reagents of claim 40, further comprising  
2   acetaminophen.

1           48. The reagents of claim 40, further comprising an  
2   acetaminophen glucuronide and/or an phenylacetylglutamine  
3   standard.

1           49. The reagents of claim 40, wherein compositions  
2   are prepared for oral administration.

1           50. The reagents of claim 40, wherein the Krebs  
2   cycle precursor is selected from the group consisting of  
3   pyruvic acid, acetic acid, acetoacetic acid, beta-  
4   hydroxybutyric acid, a Krebs cycle pathway metabolite,  
5   and mixtures thereof.

1           51. The reagents of claim 40, wherein the pH of the  
2 components of the reagent kit is from about 3 to about 7.

1           52. The reagent kit of claim 40, further comprising  
2 a buffered isotonic solution.

1           53. The reagent kit of claim 40, further comprising  
2 a buffered isotonic solution wherein the buffer comprises  
3 sodium borate and potassium cyanide.

1           54. A method for determining gluconeogenesis  
2 comprising the steps of:

3           providing a patient with a  $^{13}\text{C}$  labeled Krebs cycle  
4 precursor and  $\text{D}_2\text{O}$ ;

5           obtaining a sample a blood, urine or tissue sample from  
6 the patient;

7           measuring the  $^2\text{H}$  signal nuclear magnetic resonance  
8 spectra;

9           measuring the  $^1\text{H}$  NMR nuclear magnetic resonance spectra;

10          measuring the  $^{13}\text{C}$ -carbon nuclear magnetic resonance  
11 spectra; and

12          calculating the rate of gluconeogenesis by taking the  
13 known infusion rate of a  $^{13}\text{C}$  radiolabelled Krebs cycle

14 metabolite divided by the average fraction found in the  
15 sample over a predetermined period.

1 55. The method of claim 54, therein the  
2 predetermined time period is between about 2 to between  
3 about 3 hours.

1 56. The method of claim 54, therein the  
2 predetermined time period comprises measurements at 120,  
3 150 and 180 minutes.

1 57. The method of claim 54, wherein the patient  
2 fasts for 6-8 hours before taking the  $^{13}\text{C}$  labeled Krebs  
3 cycle precursor and  $\text{D}_2\text{O}$ .

1 58. The method of claim 54, wherein the patient is  
2 further provided with  $^{13}\text{C}$  propionate.

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